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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/454,334	12/03/99	HRUSKA K	BJCH 10041

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SENNIGER POWERS LEAVITT AND ROEDEL
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EXAMINER	
KERR, J	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/454,334	Applicant(s) Keith Hruska
	Examiner Janet M. Kerr	Group Art Unit 1633

☒ Responsive to communication(s) filed on *Dec 3, 1999* .

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *3* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *1-11* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *1-11* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____ .

☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). *6* .

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Claims 1-11 are presented for examination on the merits.

Priority

If applicant desires priority under 35 U.S.C. 119(e) based upon a previously filed copending application, specific reference to the earlier filed application must be made in the instant application. This should appear as the first sentence of the specification following the title, preferably as a separate paragraph.

Applicant should amend the specification, by inserting on line 1 of the specification, the statement, "This application claims benefit of priority to provisional application serial number 60/110,932, filed 12/05/98, and to provisional application serial number 60/111,676, filed 12/10/98, the contents of which are herein incorporated by reference.".

Should applicant amend the specification as indicated above, then the sentence "The contents of Applicant's provisional applications..." should be deleted.

Claim Objections

Claims 1, 4, 6, and 8-11 are objected to because of the following informalities: in claims 10 and 11, the phrase "a progeny" should be changed to "progeny"; in claims 1, 4, 6, and 8-11, the names of the genes/proteins associated with the abbreviations α-ENaC and α-rENaC should be explicitly stated in the first claim in which the name appears followed by the abbreviation in parentheses. In subsequent claims, the abbreviation only is sufficient. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to

make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 2-7, and 9 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 2 and 7 are directed to a transgenic non-human animal which further comprises a murine. As written the claims read on a chimeric transgenic animal. While the specification describes a transgenic mice, the specification does not provide any description of chimeric transgenic non-human animals, nor any description regarding methods of making chimeric transgenic non-human animals. The limited information provided in the specification is not deemed sufficient to reasonably convey to one skilled in the art that Applicants were in possession of transgenic non-human chimeric animals.

Claims 3 and 4 are directed to an isolated and purified nucleotide construct comprising a nucleotide sequence which has at least 80% homology with SEQ ID NO: 1 (claim 3), wherein the nucleotide sequence encodes a protein having α-ENaC activity (claim 4). The specification teaches that the term "% homology" refers to the degree of similarity between two nucleic acid sequences and may be determined by calculating the percentage of the nucleotides in the candidate sequence that are the same as the known nucleic acid sequence, after aligning the sequence and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity (see page 6, lines 8-13 of the instant application). It should be noted, however, that the specification does not teach a specific algorithm or parameters required to calculate the claimed sequence homology. For example, the necessary parameters required to calculate the claimed sequence homology, using a disclosed, given algorithm, include gap penalties and mismatch penalties. As percent homology may vary depending upon how gaps, substitutions, and sequences of unequal length are scored,

one of skill in the art would not have been able to make any particular DNA sequence at less than 100% homology, which encodes a protein having α-ENaC activity, given the limited information in the specification, without undue experimentation. Moreover, as claim 4 encompasses a nucleotide construct comprising a sequence encoding a protein having a-ENaC activity, and wherein the sequence comprises any portion of the nucleotide sequence which has at least 80% homology with SEQ ID NO: 1 and which can contain only 36 nucleotide residues in length, given the lack of description in the specification as to which nucleotide sequence having at least 80% homology with SEQ ID NO: 1, which contain only 36 nucleotide residues in length and which encodes a protein having α-ENaC activity, one of skill in the art would not be able to envision the structures of nucleic acid sequences which are embraced by the claim. As the specification does not provide a written description that would allow one of skill in the art to immediately envisage the specific structure for the claimed nucleic acid sequences, the limited information provided in the specification is not deemed sufficient to reasonably convey to one skilled in the art that Applicants were in possession of the claimed nucleic acid sequences. As the specification does not provide a written description of the nucleic acid sequences contemplated in the claimed invention, the specification also does not provide a written description of transgenic animals comprising vector constructs containing these nucleic acid sequences.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111, clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed.*" (See page 1117.) The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." (See *Vas-Cath* at page 1116).

As there is no disclosure of the claimed nucleic acid sequences, the skilled artisan cannot envision the detailed chemical structure of the encompassed nucleic acid sequences, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. Adequate written description requires more than a mere

statement that it is part of the invention and reference to a potential method of isolating it. The nucleic acid itself is required. See *Fiers v. Revel*, 25 USPQ2d 1601 at 1606 (CAFC 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016.

The limited information provided in the specification is not deemed sufficient to reasonably convey to one skilled in the art that Applicants were in possession of chimeric transgenic animals, the nucleic acid sequences discussed above, and transgenic non-human animals comprising the aforementioned nucleic acid sequences. Thus it is concluded that the written description provision of 35 U.S.C. §112, first paragraph, is not satisfied for the claimed animals and nucleic acid sequences. Applicants are reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

Claims 1-11 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claims are directed to and/or encompass transgenic non-human animals having altered expression of αENaC, chimeric transgenic non-human animals having altered expression of αENaC (specifically claims 2 and 7), methods of making transgenic non-human animals having altered expression of αENaC, isolated nucleic acid constructs comprising nucleotide sequences having at least 80% homology with SEQ ID NO: 1, which contain only 36 nucleotide residues in length and which encodes a protein having α-ENaC activity, transgenic non-human animals comprising the nucleic acid constructs, and transgenic non-human animals containing a transgene comprising a vector pKBpA which contains a nucleic acid sequence encoding α-rENaC.

The specification is non-enabling for the chimeric transgenic non-human animals having altered expression of αENaC, or transgenic non-human animals comprising the nucleic acid constructs comprising nucleotide sequences having at least 80% homology with SEQ ID NO: 1,

which contain only 36 nucleotide residues in length and which encodes a protein having α-ENaC activity as the specification does not provide sufficient guidance as how to make the nucleic acid constructs, the transgenic non-human animals comprising the constructs, or chimeric transgenic non-human animals for the reasons set forth under the written description rejection. Moreover, as the specification does not provide working examples of such animals, or any description of the phenotype displayed by such animals, the skilled artisan would not know how to use the transgenic non-human animals.

The specification is non-enabling for the claimed transgenic non-human animals as the specification only discloses a transgenic mouse which overexpresses α-rENaC (see page 8, under "Examples" of the instant application). The specification fails to provide guidance as to how to make the transgenic mouse either containing the transgene in all of the germ and somatic cells as there is no disclosure of the components of the transgene construct. With regard to the transgene construct, the specification discloses that "osteoblast cells in these transgenic mice have altered expression of stretch-activated cation channel due to the incorporation of the nucleotide construct pKBpA/α-rENaC into the genome of the animal" (see page 2, lines 11-13); that the transgene comprises a vector nucleotide sequence pKBpA wherein the stretch-activated cation channel (α-rENaC) is inserted into the pKBpA (see page 3, lines 20-23), that the pKBpA/α-rENaC animals are generated by transforming the mammals with a transgene comprising an α-rENaC cDNA or its variant inserted into a pKBpA gene and fused to a promoter such as an osteocalcin promoter (see page 6, lines 22-24). However, there is no description of the vector components such as the vector backbone and the promoter of pKBpA/α-rENaC. Thus, one of skill in the art would not be able to make this vector construct given the teachings in the specification, and use the vector construct to make the transgenic mice which requires stable integration of the vector construct. Moreover, the specification fails to provide guidance as to the method of delivery of the transgene construct only to somatic cells, such as an osteocyte or osteoblast. It is not readily apparent from the specification how to generate the transgene construct, or how to deliver the transgene

construct such that an altered level of expression of α-ENaC in the transgenic non-human animal is obtained and results in a particular phenotype.

With regard to transgenic non-human animals, *per se*, while mouse embryonic stem cells used for producing transgenic mice are readily available to the skilled artisan, the specification does not provide any guidance as to how to obtain embryonic stem cells from animals other than mice, nor does the specification disclose how to deliver a transgene construct to somatic cells, which cells should be targeted, the level of expression of the transgene required. It should also be noted that the art of producing transgenic non-human animals, *per se*, is neither routine nor predictable. At the time of filing, the state of the art is such that the generation of embryonic stem cells, i.e., cells which retain their totipotential capacity and are able to generate cells of all lineages, including germline, after being introduced into host a blastocyst, is neither routine nor predictable in species other than mice. Seamark (Reproductive Fertility and Development, 1994) discloses that totipotency for ES cell technology in many livestock species has not been demonstrated (page 6, Abstract). Similarly, Mullins *et al.* (J. Clin. Invest., 98:S37-S40, 1996) teach that while chimeric animals for several species has been produced using purported ES cells, germ line transmission of an ES cell has not been demonstrated in species other than mice (see, e.g., page S38, left column).

It should also be noted that the level of skill in the transgenic art is such that one cannot predict whether a transgene that is expressed in one mammal will also be expressed efficiently in another mammal. For example, Kappel *et al.* (Current Opinion in Biotechnology, 3:548-553, 1992) teach that while transgenes can be targeted, inherent cellular mechanism may alter the pattern of gene expression (see, e.g., page 549, right column). Similarly, Cameron (Molecular Biology, 7:253-265, 1997) teaches that

"Well-regulated transgene expression is the key to successful transgenic work, but all too often experiments are blighted by poor levels or the complete absence of expression, as well as less common problems, such as leaky expression in nontargeted tissues....". "A feature common to many transgenic experiments is the unpredictable nature of transgene expression with different transgenic lines produced with

the same construct frequently displaying different levels of expression. Further, expression levels do not correlate with the number of transgene copies integrated...". "Such copy-number-independent, integration-site-dependent expression patterns emphasize the influence of surrounding chromatin on the transgene" (see, e.g., page 256 under "Transgene Regulation and Expression")..

Thus, with respect to the unpredictability of transgene expression levels sufficient to confer a particular phenotype due to species differences and/or specific elements within a transgene construct, it would have required undue experimentation for the skilled artisan in the art to make the transgenic non-human animal as claimed. As the specification lacks guidance as to how to make the transgenic non-human animal, the specification is also non-enabling for how to use the transgenic non-human animal.

Given the lack of guidance in the specification of obtaining embryonic stem cells from species other than mice, the lack of working examples of transgenic non-human animals other than mice, and the unpredictability associated with generating transgenic non-human animals, *per se*, it would have required undue experimentation for one of skill in the art to make the transgenic non-human animal as claimed.

With regard to the transgene construct, the specification fails to teach how to make a transgene construct which when incorporated into the germ and/or somatic cells of the non-human animal, results in an altered expression of α-ENaC such that a phenotype is observed. While claim 10 recites the use of a pKBpA vector comprising a nucleic acid sequence encoding α-rENaC, the specification does not disclose the components of the pKBpA vector, a nucleic acid sequence associated with the pKBpA vector, a restriction map of the vector, or the source of the vector. One of skill in the art would not know how to make the vector such that the α-rENaC transgene can be inserted into the vector. In addition, the specification only suggests that an "osteocalcin promoter" can be used to direct expression of the nucleic acid sequence encoding α-rENaC. In view of the lack of guidance in the specification with regard to providing a vector construct containing a suitable promoter which directs expression of the α-rENaC transgene, one

of skill in the art would not be able to make the vector required to produce the transgenic non-human animal. In addition, one of skill in the art would not know the expected phenotype of such a transgenic non-human animal. Moreover, at the time of filing, the art of transgenics was known to be unpredictable with respect to the efficacy of incorporation of transgenes and the phenotypes expressed as a result of the transgene incorporation. Palmiter *et al.* (Proc. Natl. Acad. Sci, USA, 1991) teach that directed expression of any gene to any specific cell type of an animal by using established transgenic methodology is theoretically possible by combining the regulatory region(s) of a gene that is expressed in a cell-specific manner with any mRNA-encoding structural gene. Palmiter *et al.* note, however, that not all gene constructs work well; the two most common problems are inappropriate expression patterns and failure to achieve adequate expression levels (see page 478, left column, first paragraph). Wall (Theriogenology, 1996) discloses the unpredictability of transgene behavior due to factors such as position effect and unidentified control elements and may result in a lack of transgene expression or variable expression (see paragraph bridging pages 61-62). With regard to utilizing constructs designed for tissue specific expression, Colman (Am. J. Clin. Nutr., 1996) discloses that the choice of a DNA construct to be used for making a transgenic animal is important for obtaining appropriate expression. For example, Colman teaches that DNA constructs made with intronless viral or mammalian DNAs contained inappropriate or truncated regulatory sequences that did not behave in a tissue-specific way in transgenic mammals (page 640S, left column, under "Choice of DNA construct"). Taken together, the current status of the transgenic art is such that generating transgenic animals or mammals with a requisite phenotype, e.g., tissue-specific expression of a transgene, is neither routine nor predictable.

The specification is also non-enabling for vectors comprising osteocalcin promoters, as there is no teaching in the specification as to which appropriate upstream regulatory sequence(s) are needed in the promoter to obtain bone specific expression. The unpredictability of expression of a bone specific promoter, e.g., osteocalcin, is clearly demonstrated in the studies of Frenkel *et al.* (Endocrinology, 137:1080-1088, 1996). Frenkel *et al.* examined a series of osteocalcin

promoter constructs comprising anywhere from 108 nucleotides to 1097 nucleotides of upstream regulatory sequence (see page 1081, Figure 1). Analysis of the expression of the constructs in ROS cells, i.e., osteoblast-like cells, indicated that the promoter activity was variable not only with respect to the amount of upstream sequence in the construct, but also variable with respect to whether the construct was stably integrated into cells or was transiently transfected into the cells (see page 1083, Figure 3C for example). Thus, not only will the length of the upstream sequence impact on expression of the polynucleotide, the manner in which the polynucleotide interacts with the host cell will impact on expression of the polynucleotide as well. With regard to the inducibility of the osteocalcin promoter, McCabe *et al.* (Endocrinology, 137:4398-4408, 1996) demonstrate that different regions of the osteocalcin promoter are important for mediating the repressor or enhancer effect of AP-1 coexpression. For example, when a region containing the VDRE is deleted, repression by c-Fos and c-Jun coexpression is lost, even though other putative AP-1 binding sites, e.g, the TGRE and OC box, remain present in the construct. When the TGRE is deleted, the enhancer effect of Fra-2 and Jun D coexpression is lost, implicating this site in mediating enhancer activity (see, e.g., page 4406, right column, first full paragraph). Clearly, the presence or absence of regulatory elements will affect promoter activity. Furthermore, Clemens *et al.* (J. Bone and Mineral Research, 12:1570-1576, 1997) disclose that a human osteocalcin genomic fragment containing 4 kb of upstream sequence, all four exons, and 15 kb of downstream sequence (see page 1571, left column, under "Generation of transgenic mice"), when introduced into mice, is upregulated upon administration of $1,25(OH)_2D_3$, whereas the endogenous mouse osteocalcin gene is unresponsive to $1,25(OH)_2D_3$ (see, e.g., page 1572-1573, under "Effect of $1,25(OH)_2D_3$ on mouse and human osteocalcin *in vivo*", and page 1575, left column, first full paragraph). Clearly, the activity and inducibility of the osteocalcin promoter is not predictable as numerous variables including length of the promoter region, extrachromosomal or chromosomal localization of the exogenous promoter, inclusion or exclusion of regulatory elements, and differential species responses to trans-acting factors, affect promoter activity.

In view of the limited guidance in the specification and the unpredictability in the art of transgenics, it would require undue experimentation for the skilled artisan to make the claimed transgene constructs and use the transgene constructs in producing transgenic non-human animals. As the specification is non-enabling for producing the transgenic non-human animals, the specification is also non-enabling for using the transgenic non-human animals in methods for producing progeny.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-11 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is vague and indefinite for the following reasons: the phrase "nucleotide construct capable of altered expression of α-ENaC" renders the claim vague and indefinite because it is unclear what type of altered expression is intended, i.e., over-expression, under-expression, no expression, to what extent the expression is altered, and whether the altered expression is relative to a wild-type littermate or to some other "control" transgenic or non-transgenic non-human animal; the phrase is also confusing because it is unclear how a nucleotide construct can be capable of altered expression and under what conditions the nucleotide construct has the capability of altered expression. The claim, as written, is vague, indefinite, and confusing.

Claims 2 and 7 are rendered vague and indefinite by the phrase "wherein the animal further comprises a murine" because it is unclear if applicant intended a "chimeric animal" or if applicant intended that the transgenic non-human animal is murine.

Claims 3 and 5 are rendered vague and indefinite by the phrase "which is at least 36 nucleotide residues in length" as it is unclear if applicant intended at least 36 consecutive

nucleotide residues in length or if applicant intended any possible combination of 36 nucleotide residues which are present in the sequence.

Claim 4 is rendered vague and indefinite by the phrase "said nucleotide sequence" because it is unclear if applicant intended the nucleotide sequence of any one of a), b), or c) or if applicant intended only one particular sequence.

Claim 8 is rendered vague and indefinite by the phrase "altered expression of α-rENaC" (both occurrences) because it is unclear what type of altered expression is intended, i.e., over-expression, under-expression, no expression, to what extent the expression is altered, and whether the altered expression is relative to a wild-type littermate or to some other "control" transgenic or non-transgenic non-human animal; the phrase "providing a vector construct containing a transgenic encoding a protein" is confusing because it is unclear how a vector construct can contain a transgenic, did applicant intend a "transgene"?.

Claim 9 is rendered vague and indefinite or the following reasons: the phrase "altered expression of stretch-activated cation channel" is vague and indefinite because it is unclear what type of altered expression is intended, i.e., over-expression, under-expression, no expression, to what extent the expression is altered, and whether the altered expression is relative to a wild-type littermate or to some other "control" transgenic or non-transgenic non-human animal; the phrase "cells responsive to osteocalcin" is vague and indefinite because neither the claim nor the specification defines which cells are responsive to osteocalcin or what type of responses are observed in such cells, thus it is unclear which cells are intended and how the cells respond to osteocalcin; the phrase "the vector nucleotide sequence pKBpA" is vague and indefinite as neither the specification nor the claim defines what nucleotide sequence is encompassed in pKBpA with the exception of the α-rENaC nucleotide sequence; the phrase "the stably integrated transgene" lacks antecedent basis as there is no recitation of a stable integration in the previous method steps; the phrase "has the ability to express" as it is unclear if in fact the transgene is expressed or under what conditions the transgene is able to express; it is unclear how introducing a transgene into an osteocyte results in transgene expression in osteoblasts; and the phrase "wherein the stretch-

activated cation channel (a-rENaC) is inserted into the pKBpA" is confusing because, as written, a protein is being inserted into pKBpA rather than a nucleotide sequence which encodes α-rENaC. Clarification is requested.

Claims 10 and 11 are rendered vague and indefinite by the phrase "altered expression of stretch-activated cation channel" is vague and indefinite because it is unclear what type of altered expression is intended, i.e., over-expression, under-expression, no expression, to what extent the expression is altered, and whether the altered expression is relative to a wild-type littermate or to some other "control" transgenic or non-transgenic non-human animal; the phrase "derived from" is confusing because it is unclear what type of derivation is intended, did applicant intend "obtained from"?

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

Claims 1, 2, and 5-7 are rejected under 35 U.S.C. 102(b) as being anticipated by Hummler *et al.* (Experientia, 50:A40, Abstract #S12-13, 1994) or alternatively, over Hummler *et al.* (Experientia, 51:A7, Abstract #S03-15, 1995).

Although the specification does not provide sufficient written description or an enabling disclosure with regard to making a transgene construct comprising a CMV promoter operatively linked to a nucleic acid sequence encoding ENaC for use in generating a transgenic mouse, the breadth of the claims, as written, encompass a transgenic mouse produced by introduction of a transgene construct comprising a CMV promoter operatively linked to a nucleic acid sequence encoding ENaC. As such, the teachings of Hummler *et al.*, as discussed below, anticipate the claimed transgenic non-human animals.

Hummler *et al.* teach transgenic constructs in which the α, β and γrENac are linked to the human CMV promoter, and the generation of transgenic lines of mice which express the transgene in different tissues (see the abstract of either reference). As the transgenic mice of Hummler *et al.* have altered expression of rENaC, the references of Hummler *et al.* anticipate the claimed invention.

Claims 1, 2, and 5-7 are rejected under 35 U.S.C. 102(b) as being anticipated by Hummler *et al.* (Proc. Natl. Acad. Sci. USA, 94:11710-11715, October, 1997).

Although the specification does not provide sufficient written description or an enabling disclosure with regard to making a transgene construct comprising a CMV promoter operatively linked to a nucleic acid sequence encoding αENaC for use in generating a transgenic mouse, the breadth of the claims, as written, encompass a transgenic mouse produced by introduction of a transgene construct comprising a CMV promoter operatively linked to a nucleic acid sequence encoding αENaC. As such, the teachings of Hummler *et al.*, as discussed below, anticipate the claimed transgenic non-human animals.

Hummler *et al.* disclose a method of generating transgenic mice carrying the α subunit of ENaC under the control of an ubiquitously and constitutively expressed cytomegalovirus promoter on an αENaC knockout background by breeding homozygous and heterozygous transgenic mutant mice (see page 11710, abstract and right column, last paragraph, and page 11711, under Materials and Methods, Figure 1). Results from RT-PCR analyses indicate that

introduction of the transgene construct into the αENaC knockout mice resulted in expression of αENaC (see page 11710, abstract and right column, last paragraph, and page 11711, under Materials and Methods, Figure 1). Thus, the generated mice necessarily have altered expression of αENaC.

Claims 1, 2, 5, 7, and 11 are rejected under 35 U.S.C. 102(b) as being anticipated by Hummler *et al.* (Nature Genetics, 12:325-328, 1996).

Although the specification does not provide sufficient written description or an enabling disclosure with regard to making an ENaC knock-out mouse, the breadth of the claims, as written, encompass such a mouse in that the claims recite "altered expression of ENaC" which can be interpreted as "no expression". As such, the teachings of Hummler *et al.*, as discussed below, anticipate the claimed transgenic non-human animals.

Hummler *et al.* teach the generation of homozygous αENaC(-/-) mice produced by inactivation of the murine αENaC gene locus via homologous recombination by deleting part of the amino-terminal segment of the protein, including the first transmembrane domain and part of the large ectocytoplasmic loop, and mating mice heterozygous for αENaC to obtain homozygous αENaC(-/-) mice. Results of RT-PCR analyses of tissues, such as kidney and lung, indicate the lack of expression of the αENaC transcript in the homozygotes. Thus, the homozygous αENaC(-/-) mice necessarily have altered expression of αENaC. As Hummler *et al.* teach methods of making trangenic mice and transgenic mice comprising a construct capable of altered expression of α-ENaC, in which the construct comprises any portion of a nucleotide sequence which has at least 80% homology with SEQ ID NO: 1, and which is at least 36 nucleotide residues in length, the reference of Hummler *et al.* anticipates the claimed invention.

Claims 3 and 4 are rejected under 35 U.S.C. 102(b) as being anticipated by Canessa *et al.* (Nature, 361:467-470, 1993).

Canessa *et al.* teach a nucleotide sequence which has 100% identity to SEQ ID NO:1 of the instant invention (compare base pairs 1-3117 of the database (Db) sequence of Canessa *et al.* to base pairs 1-3117 of the query sequence (Qy) of SEQ ID NO:1 of the attached sequence comparison, under "Result 1", GenBank Accession #X70497), and thus has at least 36 nucleotide residues in length which are identical to those of SEQ ID NO: 1, has at least 80% homology to SEQ ID NO:1, and necessarily encodes a protein having a-ENaC activity. As the sequence of Canessa *et al.* meets the limitations of the claims, the sequence of Canessa *et al.* anticipates the claimed invention.

Claims 3 and 4 are rejected under 35 U.S.C. 102(b) as being anticipated by Lingueglia *et al.* (FEBS Lett., 318:95-99, 1993).

Lingueglia *et al.* teach a nucleotide sequence which has 89.7% identity to SEQ ID NO:1 of the instant invention (compare base pairs 2-3081 of the database (Db) sequence of Lingueglia *et al.* to base pairs 27-3104 of the query sequence (Qy) of SEQ ID NO:1 of the attached sequence comparison, under "Result 2", GenBank Accession #X70521), and thus has at least 36 nucleotide residues in length which are identical to those of SEQ ID NO: 1, has at least 80% homology to SEQ ID NO:1, and necessarily encodes a protein having a-ENaC activity barring evidence to the contrary. As the sequence of Lingueglia *et al.* meets the limitations of the claims, the sequence of Lingueglia *et al.* anticipates the claimed invention.

Claims 3 and 4 are rejected under 35 U.S.C. 102(b) as being anticipated by Kreutz *et al.* Hypertension, 29:131-136, January, 1997).

Kreutz *et al.* teach a nucleotide sequence comprising at least 36 nucleotide residues in length which are identical to those of SEQ ID NO: 1 of the instant application (compare for example, base pairs 1-1801 of the database (Db) sequence of Kreutz *et al.* to base pairs 47-1847 of the query sequence (Qy) of SEQ ID NO:1 of the attached sequence comparison under "Result 3", GenBank Accession #U54700, and see page 134, left column, second line of text of the

reference of Kreutz *et al.*, or alternatively, compare for example, base pairs 301-1801 of the database (Db) sequence of Kreutz *et al.* to base pairs 347-1847 of the query sequence (Qy) of SEQ ID NO:1 of the attached sequence comparison under "Result 4", GenBank Accession #U54699, and see page 134, left column, first line of text of the reference of Kreutz *et al.*). As the sequence of Kreutz *et al.* meets the limitations of the claim in that the sequence comprises a portion of the nucleotide sequence or complement of SEQ ID NO:1 which is at least 36 nucleotide residues in length (part a of claim 3), and which necessarily encodes a protein having α-ENaC activity barring evidence to the contrary, the isolated nucleotide sequence of Kreutz *et al.* anticipates the claimed invention.

Claim 3 is rejected under 35 U.S.C. 102(e) as being anticipated by Li *et al.* (U.S. Patent No. 5,693,756, 12/2/97, effective filing date of 1/23/95).

Li *et al.* teach a nucleotide sequence comprising at least 36 nucleotide residues in length which are identical to those of SEQ ID NO: 1 of the instant application (compare base pairs 1-45 of the database (Db) sequence of Li *et al.* to base pairs 1134-1178 of the query sequence (Qy) of SEQ ID NO:1 of the attached sequence comparison under "Result 13", GenBank Accession #T99077, and see col. 6, lines 66-67 of the patent of Li *et al.*, or alternatively, compare base pairs 1-45 of the database (Db) sequence of Li *et al.* to base pairs 1498-1542 of the query sequence (Qy) of SEQ ID NO:1 of the attached sequence comparison under "Result 14", GenBank Accession #T99078, and see col. 7, lines 2-3 of the patent of Li *et al.*). As the sequence of Li *et al.* meets the limitations of the claim in that the sequence comprises a portion of the nucleotide sequence or complement of SEQ ID NO:1 which is at least 36 nucleotide residues in length (part a of claim 3), the isolated nucleotide sequence of Li *et al.* anticipates the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 8 and 10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hummler *et al.* (Proc. Natl. Acad. Sci. USA, 94:11710-11715, October, 1997) taken with Kizer *et al.* (Proc. Natl. Acad. Sci. USA, 94:1013-1018, February, 1997).

Although the specification does not provide sufficient written description or an enabling disclosure with regard to making a transgene construct comprising a CMV promoter operatively linked to a nucleic acid sequence encoding αENaC for use in generating a transgenic mouse, the breadth of the claims, as written, encompass methods of breeding transgenic mice to produce mice of different genotypes, and a transgenic mouse produced by introduction of a transgene construct comprising a CMV promoter operatively linked to a nucleic acid sequence encoding αENaC. As such, the teachings of Hummler *et al.*, taken with Kizer et al., as discussed below, render the claimed methods obvious.

Hummler *et al.* disclose a method of generating transgenic mice carrying the α subunit of ENaC under the control of an ubiquitously and constitutively expressed cytomegalovirus promoter on an αENaC knockout background by breeding homozygous and heterozygous transgenic mutant mice (see page 11710, abstract and right column, last paragraph, and page 11711, under Materials and Methods, Figure 1). Results from RT-PCR analyses indicate that introduction of the transgene construct into the αENaC knockout mice resulted in expression of αENaC (see page 11710, abstract and right column, last paragraph, and page 11711, under Materials and Methods, Figure 1). Thus, the generated mice necessarily have altered expression of αENaC.

Hummler *et al.* do not disclose that the transgenic mice have altered expression of α-rENaC in osteoblasts. However, Kizer *et al.* disclose transfecting osteoblast-like cell lines with the expression vector pCEP4 which contains, as an insert, the full-length coding region of α-

rENaC. (It is known that pCEP4, obtained from Invitrogen, contains the CMV promoter). The transfected cells express α-rENaC (see, e.g., page 1014, left column, under "Preparation of Transfection Vector", page 1015, right column, last paragraph, and Figure 3).

In view of the teachings of Kizer *et al.* that osteoblast-like cells transfected with an expression construct comprising the CMV promoter operatively linked to α-rENaC express α-rENaC, and in view of the teachings of Hummler *et al.* that the transgene construct, used to generate transgenic mice comprises the CMV promoter operatively linked to α-rENaC, and is expressed in different tissues in mice, one of ordinary skill in the art would have had a high expectation that the transgenic mice of Hummler *et al.* express the α-rENaC transgene in osteoblasts barring evidence to the contrary.

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to make a transgenic animal which expresses α-rENaC in osteoblasts by stably integrating into the genome of a mouse a transgene construct comprising the CMV promoter operatively linked to α-rENaC, and breeding such mice to obtain transgenic mice with different genotypes, as Hummler *et al.* teach that the transgenic mice express α-rENaC in different tissues. In view of the teachings of Kizer *et al.* that osteoblast-like cells transfected with an expression vector comprising the CMV promoter operatively linked to a nucleic acid sequence encoding α-rENaC express α-rENaC, one of skill in the art would have had a high expectation that a similar construct would have been expressed in osteoblasts in transgenic mice containing the expression vector.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear, and convincing evidence to the contrary.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Janet M. Kerr whose telephone number is (703) 305-4055. Should the examiner be unavailable, inquiries should be directed to John LeGuyader, Supervisory Primary Examiner of Art Unit 1633, at (703) 308-0447. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 305-7401. Any inquiry of a general nature or relating to the status of this application should be directed to the Group 1600 receptionist whose telephone number is (703) 308-0196.

The Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633.

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